

FILE 'CAPLUS' ENTERED AT 14:43:11 ON 29 MAR 2004

=> E NAKANISHI Y/AU

=> S E3,E66

216 "NAKANISHI Y"/AU

26 "NAKANISHI YUJI"/AU

L1 242 ("NAKANISHI Y"/AU OR "NAKANISHI YUJI"/AU)

=> E KARIYA K/AU

=> S E3,E24,E25

23 "KARIYA K"/AU

1 "KARIYA KIN YA"/AU

20 "KARIYA KINYA"/AU

L2 44 ("KARIYA K"/AU OR "KARIYA KIN YA"/AU OR "KARIYA KINYA"/AU)

=> S LIPASE

41532 LIPASE

8242 LIPASES

L3 42773 LIPASE

(LIPASE OR LIPASES)

=> S L1 AND L2 AND L3

L4 1 L1 AND L2 AND L3

=> D CBIB ABS

L4 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN

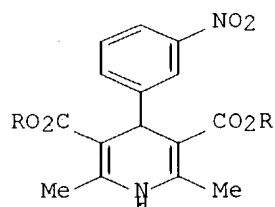
1995:382025 Document No. 122:239505 Inversion of enantioselectivity in

hydrolysis of 1,4-dihydropyridines by point mutation of **lipase**

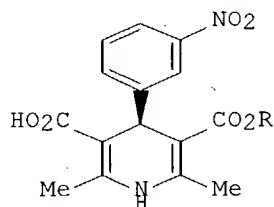
PS. Hirose, Yoshihiko; **Kariya, Kinya; Nakanishi, Yuji**

; Kurono, Yoshiaki; Achiwa, Kazuo (Cent. Res. Lab., Amano Pharmaceutical Co., Ltd., Nishikasugai, 481, Japan). Tetrahedron Letters, 36(7), 1063-6 (English) 1995. CODEN: TELEAY. ISSN: 0040-4039. Publisher: Elsevier.

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I



II

AB A mutant **lipase** differing in three amino acids produced alternate enantiomers when reacted with 1,4-dihydropyridinedicarboxylates. Thus, **lipase** PS reacted with dihydropyridinecarboxylates I (R = CH₂OCOEt, CH₂OCOCMe₃) to give the (R) monocarboxylic acids II, whereas a mutant **lipase** produced the (S) enantiomer.

=> S STEROSSEL?

L5 2 STEROSSEL?

=> S STEREOSEL?

L6 75296 STEREOSEL?

=> S (L5,L6) and (L1 OR L2)

L7 4 ((L5 OR L6)) AND (L1 OR L2)

=> D 1-4 CBIB ABS

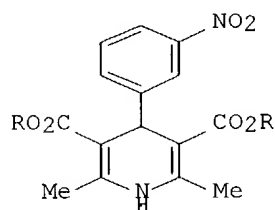
L7 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN

1995:382025 Document No. 122:239505 Inversion of enantioselectivity in hydrolysis of 1,4-dihydropyridines by point mutation of lipase PS.

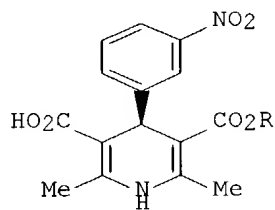
Hirose, Yoshihiko; **Kariya, Kinya**; **Nakanishi, Yuji**;

Kurono, Yoshiaki; Achiwa, Kazuo (Cent. Res. Lab., Amano Pharmaceutical Co., Ltd., Nishikasugai, 481, Japan). Tetrahedron Letters, 36(7), 1063-6 (English) 1995. CODEN: TELEAY. ISSN: 0040-4039. Publisher: Elsevier.

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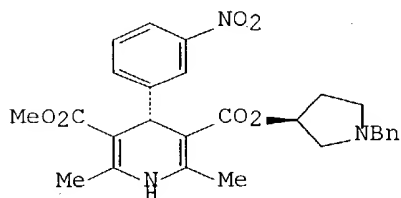
AB A mutant lipase differing in three amino acids produced alternate enantiomers when reacted with 1,4-dihydropyridinedicarboxylates. Thus, lipase PS reacted with dihydropyridinecarboxylates I (R = CH₂OCOEt, CH₂OCOCMe₃) to give the (R) monocarboxylic acids II, whereas a mutant lipase produced the (S) enantiomer.

L7 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN

1994:217203 Document No. 120:217203 Carbamoylmethyl group as an activated group in protease- and base-catalyzed transesterification of 1,4-dihydropyridines: a novel asymmetric synthesis of valnidipine.

Hirose, Yoshihiko; **Kariya, Kinya**; Sasaki, Ikuharu; Kurono, Yoshiaki; Achiwa, Kazuo (Cent. Res. Lab., Amano Pharm. Co., Ltd., Nishikasugai, 481, Japan). Tetrahedron Letters, 34(37), 5915-18 (English) 1993. CODEN: TELEAY. ISSN: 0040-4039. OTHER SOURCES: CASREACT 120:217203.

GI



I

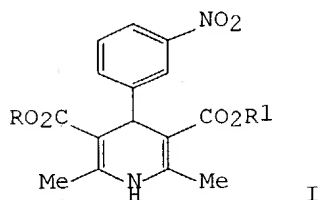
AB The first protease-catalyzed enantioselective transesterification of 1,4-dihydropyridine-3,5-dicarboxylates in an aqueous solution was developed with

high optical purity. Carbamoylmethyl ester group was enantioselectively transesterified with (S)-N-benzyl-3-pyrrolidinol by the protease ; successive base-catalyzed transesterification proceeded smoothly to give the chiral drug, valnidipine (I), in a good yield.

L7 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN

1993:408641 Document No. 119:8641 Drastic solvent effect on lipase-catalyzed enantioselective hydrolysis of prochiral 1,4-dihydropyridines. Hirose, Yoshihiko; **Kariya, Kinya**; Sasaki, Ikuharu; Kurono, Yoshiaki; Ebiike, Hirosato; Achiwa, Kazuo (Cent. Res. Lab., Amano Pharm. Co., Ltd., Aichi, 481, Japan). Tetrahedron Letters, 33(47), 7157-60 (English) 1992. CODEN: TELEAY. ISSN: 0040-4039.

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AB Two enantiomers of 1,4-dihydropyridine compds. have been obtained with high enantiomeric purity by lipase-catalyzed hydrolysis in organic solvent saturated with water. Enantioselectivity of this reaction is dependent on the solvent used. Thus, the lipase-catalyzed hydrolysis of dihydropyridinedicarboxylates I ($R = R_1 = \text{CH}_2\text{O}_2\text{CR}_2$; $R_2 = \text{Me, Et, Pr, CHMe}_2, \text{CMe}_3$) in $(\text{Me}_2\text{CH})_2\text{O}$ saturated with water gave (S)-enantiomers of the monoesters I ($R = \text{H, } R_1 \text{ as above}$), whereas, hydrolysis in cyclohexane saturated with water gave the (R)-enantiomers of the monoesters I ($R = \text{H, } R_1 \text{ as above}$).

L7 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN

1992:419802 Document No. 117:19802 **Stereoselective** conjugation of a uricosuric diuretic with glutathione by glutathione transferase 3-3. **Kariya, K.**; Okumura, H.; Lee, E.; Hirata, M. (Fac. Pharm. Sci., Kobe-Gakuin Univ., Kobe, 651-21, Japan). Xenobiotica, 22(3), 319-23 (English) 1992. CODEN: XENOBH. ISSN: 0049-8254.

AB The activity of glutathione (GSH) transferases in rat liver cytosol was inhibited by the (-) enantiomer of a uricosuric diuretic (6,7-dichloro-5[n,n-dimethylsulfamoyl]-2,3-dihydrobenzofuran-2-carboxylic acid, DBCA) in a concentration-dependent manner. Although the DBCA (+) enantiomer inhibited the activity of liver cytosol GSH transferase, it was less effective. Among four purified GSH transferase isoenzymes obtained from rat liver cytosol, isoenzyme 3-3 showed **stereoselective** interactions with the enantiomers of DBCA. This isoenzyme most actively and preferentially catalyzed the transfer of GSH to DBCA (-) enantiomer.

=> S MUTATION

198958 MUTATION

128238 MUTATIONS

L8 246970 MUTATION

(MUTATION OR MUTATIONS)

=> S L6 AND L8
L9 112 L6 AND L8

=> S L6(3A)L8
L10 9 L6(3A)L8

=> S L6(6A)L8
L11 12 L6(6A)L8

=> S L11 NOT L7
L12 12 L11 NOT L7

=> D 1-12 CBIB ABS

L12 ANSWER 1 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
2003:911989 Document No. 140:196160 Efficient expression and mutation of
avidin and streptavidin as host proteins for enantioselective catalysis.
Zocchi, Andrea; Humbert, Nicolas; Berta, Temugin; Ward, Thomas R.
(Institute of Chemistry, University of Neuchatel, Neuchatel, CH-2007,
Switz.). Chimia, 57(10), 589-592 (English) 2003. CODEN: CHIMAD. ISSN:
0009-4293. Publisher: Swiss Chemical Society.

AB Avidin and structurally related streptavidin are attractive host proteins for
the creation of artificial metalloenzymes displaying features reminiscent both
of homogeneous catalysts and enzymes. The main advantages are that both
proteins have been cloned and expressed in several organisms and possess a
deep hydrophobic binding pocket capable of hosting biotinylated catalyst
precursors. An optimized artificial avidin gene in *Pichia pastoris* is
expressed. The high level of active protein produced in the extracellular
medium is suitable for the performance of high-throughput screening in 96-well
plate format. Biol. active recombinant streptavidin is expressed in *E. coli*.
Mutations have been introduced both in avidin and streptavidin genes and both
wild type and mutated proteins have been utilized to explore the role of the
second coordination sphere in enantioselective catalysis.

L12 ANSWER 2 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN
2003:420313 Document No. 139:207643. Point mutations at L1280 in Nav1.4
channel D3-S6 modulate binding affinity and stereoselectivity of
bupivacaine enantiomers. Nau, Carla; Wang, Sho-Ya; Wang, Ging Kuo
(Department of Anesthesiology, Friedrich-Alexander-University
Erlangen-Nuremberg, Erlangen, Germany). Molecular Pharmacology, 63(6),
1398-1406 (English) 2003. CODEN: MOPMA3. ISSN: 0026-895X. Publisher:
American Society for Pharmacology and Experimental Therapeutics.

AB Local anesthetics (LAs) block voltage-gated sodium channels. Parts of the LA
binding site are located in the pore-lining transmembrane segments 6 of
domains 1, 3, and 4 (D1-S6, D3-S6, D4-S6). We suggested previously that
residue N434 in D1-S6 interacts directly with bupivacaine enantiomers in
inactivated channels because side-chain properties of different residues
substituted at N434 correlated with changes in blocking potencies of
bupivacaine enantiomers. Furthermore, **mutation** N434R exhibited significant
stereoselectivity for block of inactivated channels that resulted from a
selective decrease in block by S(-)-bupivacaine. In the present study, we
analyzed the role of residue L1280 in D3-S6 of the rat skeletal muscle Nav1.4
channel in interactions with the enantiomers of bupivacaine. We substituted
native leucine at L1280 with amino acids of different physicochem. properties.
Wild-type and mutant channels were expressed transiently in human embryonic
kidney 293t cells and were investigated under whole-cell voltage clamp. Block
of resting mutant channels by bupivacaine enantiomers revealed little
difference compared with wild-type channels. Block of inactivated channels
was increased in a mutation containing an aromatic group (L1280W) and

decreased in mutations containing a pos. charge (L1280K, L1280R). Surprisingly, mutants L1280E, L1280N, L1280Q, and L1280R exhibited significant stereoselectivity for block of inactivated channels. More surprisingly, stereoselectivity resulted from a selective decrease in block by R(+)-bupivacaine, in contrast to mutation N434R in D1-S6. We propose that in inactivated channels, residues L1280 in D3-S6 and N434 in D1-S6 interact directly with LAs and thereby face each other in the ion-conducting pore.

L12 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

2002:102070 Document No. 136:336960 Active Site Mutations of Cytochrome P450cam Alter the Binding, Coupling, and Oxidation of the Foreign Substrates (R)- and (S)-2-Ethylhexanol. French, Kevin J.; Rock, Dan A.; Rock, Denise A.; Manchester, John I.; Goldstein, Barry M.; Jones, Jeffrey P. (Toxicology Training Program, Department of Environmental Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY, 14642, USA). Archives of Biochemistry and Biophysics, 398(2), 188-197 (English) 2002. CODEN: ABBIA4. ISSN: 0003-9861. Publisher: Academic Press.

AB Three factors are of primary importance with respect to designing efficient P 450 biocatalysts. (1) The substrate must be oxidized at a significant rate. (2) The regioselectivity must heavily favor the desired product. (3) The enzyme must use the majority of the reducing equivalent from NADH or NADPH to produce product. The reaction we chose to study was oxidation of 2-ethylhexanol to 2-ethylhexanoic acid by P450cam. We examined four active site mutations: F87W, Y96W, T185F, and L244A. The mutations were chosen to improve 2-ethylhexanoic acid production by decreasing active site volume, increasing active site hydrophobicity, and improving stereoselectivity. The F87W and Y96W mutations improved regioselectivity, giving almost exclusively the desired product. The T185F mutation improved coupling of NADH to product formation. The L244A **mutation** altered the **stereoselectivity** of 2-ethylhexanoic acid production. These results indicate that active site mutations of P450cam can alter catalysis of 2-ethylhexanol. (c) 2002 Academic Press.

L12 ANSWER 4 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

2002:86743 Document No. 136:379471 Binding characteristics of cetirizine and levocetirizine to human H1 histamine receptors: contribution of Lys191 and Thr194. Gillard, Michel; Van Der Perren, Christy; Moguilevsky, Nicole; Massingham, Roy; Chatelain, Pierre (UCB S.A. Pharma Sector, In Vitro Pharmacology, Braine l'Alleud, Belg.). Molecular Pharmacology, 61(2), 391-399 (English) 2002. CODEN: MOPMA3. ISSN: 0026-895X. Publisher: American Society for Pharmacology and Experimental Therapeutics.

AB Competition expts. with [3H]mepyramine showed that cetirizine and its enantiomers, levocetirizine and (S)-cetirizine, bound with high affinity and stereoselectivity to human H1 histamine receptors (Ki values of 6, 3, and 100 nM, resp.). Cetirizine and levocetirizine were 600-fold more selective for H1 receptors compared with a panel of receptors and channels. Binding results indicated that the interaction between cetirizine, its enantiomers, and histamine is compatible with a competitive behavior, in contrast with the noncompetitive profile of cetirizine and levocetirizine observed in isolated organs. Binding kinetics provided a suitable explanation for this observation, because levocetirizine dissociated from H1 receptors with a half-time of 142 min; that of (S)-cetirizine was only 6 min, implying that the former could act as a pseudo-irreversible antagonist in functional studies. The carboxylic function of levocetirizine seemed responsible for its long dissociation time. Indeed, hydroxyl or Me ester analogs dissociated more rapidly from H1 receptors, with half-times of 31 min and 7 min, resp. The importance of the carboxylic function of levocetirizine for the interaction

with the H1 receptor was further supported by the results from the mutation of Lys191 to Ala191. This mutation decreased the dissociation half-time of levocetirizine from 142 to 13 min and reduced its affinity from 3 to 12 nM, whereas the affinity and dissociation kinetics of hydroxyl and Me ester analogs were hardly affected. The **mutation** of Thr194 reduced the binding **stereoselectivity** by selectively enhancing the affinity of the distomer.

L12 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

2001:481447 Document No. 135:223315 DNA Polymerase β : Pre-Steady-State Kinetic Analyses of dATP α S Stereoselectivity and Alteration of the Stereoselectivity by Various Metal Ions and by Site-Directed Mutagenesis. Liu, Jia; Tsai, Ming-Daw (Departments of Biochemistry and Chemistry, Ohio State Biochemistry Program and Protein Research Group, The Ohio State University, Columbus, OH, 43210, USA). Biochemistry, 40(30), 9014-9022 (English) 2001. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.

AB The first pre-steady-state kinetic anal. of the stereoselectivity of a DNA polymerase, Pol β from rat brain, toward Rp and Sp isomers of dATP α S, and alteration of the stereoselectivity by various metal ions and by site-directed mutagenesis are reported. Diastereomers of dATP α S were synthesized by enzymic methods to >98% purity. The rate of polymerization (k_{pol}) and the apparent dissociation constant ($K_{d,app}$) were measured with dATP, Rp-dATP α S, and Sp-dATP α S in the presence of Mg $^{2+}$, Mn $^{2+}$, or Cd $^{2+}$. The results indicate that wild type (WT) polymerase (Pol) β can incorporate both Sp- and Rp-dATP α S in the presence of Mg $^{2+}$, but Sp is the preferred isomer. The stereoselectivity, defined as $(k_{pol}/K_d)_{Sp}/(k_{pol}/K_d)_{Rp}$ (abbreviated Sp/Rp ratio), is 57.5 in the presence of Mg $^{2+}$. When Mg $^{2+}$ was substituted with Mn $^{2+}$ and Cd $^{2+}$, the Sp/Rp ratio decreased to 7.6 and 21, resp. These results are discussed in relation to the crystal structures of various Pol β complexes, as well as previous steady-state kinetic studies of other DNA polymerases. In addition, the D276R mutant was designed to introduce a potential extra hydrogen bonding interaction between the arginine side chain and the pro-Sp oxygen of the α -phosphate of dNTP. The kinetic data of the D276R mutant showed a pronounced relaxation of stereoselectivity of dATP α S (Sp/Rp ratio = 1.5, 3.7, and 1.5 for Mg $^{2+}$, Mn $^{2+}$, and Cd $^{2+}$, resp.). Furthermore, the D276R mutant showed a 5-fold enhanced reactivity toward Rp-dATP α S relative to WT Pol β , suggesting that this mutant Pol β can be used to incorporate Rp-dNTP α S into DNA oligomers.

L12 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

2001:466971 Document No. 135:238431 Mutation of cysteine-295 to alanine in secondary alcohol dehydrogenase from Thermoanaerobacter ethanolicus affects the enantioselectivity and substrate specificity of ketone reductions. Heiss, C.; Laivenieks, M.; Zeikus, J. G.; Phillips, R. S. (Department of Chemistry and Molecular Biology, University of Georgia, Athens, GA, 30602-2556, USA). Bioorganic & Medicinal Chemistry, 9(7), 1659-1666 (English) 2001. CODEN: BMECEP. ISSN: 0968-0896. OTHER SOURCES: CASREACT 135:238431. Publisher: Elsevier Science Ltd..

AB The mutation of Cys-295 to alanine in Thermoanaerobacter ethanolicus secondary alc. dehydrogenase (SADH) was performed to give C295A SADH, on the basis of mol. modeling studies utilizing the x-ray crystal structure coordinates of the highly homologous T. brockii secondary alc. dehydrogenase (1YKF.PDB). This mutant SADH has activity for 2-propanol comparable to wild-type SADH. However, the C295A mutation was found to cause a significant shift of enantioselectivity toward the (S)-configuration in the reduction of some ethynylketones to the corresponding chiral propargyl alcs. This result confirms our prediction that Cys-295 is part of a small alkyl group binding

pocket whose size det. the binding orientation of ketone substrates, and, hence, the stereochem. configuration of the product alc. Furthermore, C295A SADH has much higher activity towards t-Bu and some α -branched ketones than does wild-type SADH. The C295A mutation does not affect the thioester reductase activity of SADH. The broader substrate specificity and altered stereoselectivity for C295A SADH make it a potentially useful tool for asym. redns. The C295A mutation of SADH was found to cause a significant shift of enantioselectivity toward the (S)-configuration in the reduction of some ethynylketones. C295A SADH has much higher activity towards t-Bu and some α -branched ketones than does wild-type SADH.

L12 ANSWER 7 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

1998:601989 Document No. 129:312661 Rational design of *Rhizopus oryzae* lipase with modified stereoselectivity toward triacylglycerols. Scheib, H.; Pleiss, J.; Stadler, P.; Kovac, A.; Potthoff, A. P.; Haalck, L.; Spener, F.; Paltauf, F.; Schmid, R. D. (Institute of Technical Biochemistry, University of Stuttgart, Stuttgart, D-70569, Germany). Protein Engineering, 11(8), 675-682 (English) 1998. CODEN: PRENE9. ISSN: 0269-2139. Publisher: Oxford University Press.

AB The binding site of sn-1(3)-regioselective *Rhizopus oryzae* lipase (ROL) has been engineered to change the stereoselectivity of hydrolysis of triacylglycerol substrates and analogs. Two types of prochiral triacylglycerols were considered: "flexible" substrates with ether, benzylether or ester groups, and "rigid" substrates with amide or Ph groups, resp., in the sn-2 position. The mol. basis of sn-1(3) stereoselectivity of ROL was investigated by modeling the interactions between substrates and ROL, and the model was confirmed by exptl. determination of the stereoselectivity of wild-type and mutated ROL. For the substrates, the following rules were derived: (i) stereopreference of ROL toward triacylglycerols depends on the substrate structure. Substrates with "flexible" sn-2 substituents are preferably hydrolyzed at sn-1, "rigid" substrates at sn-3. (ii) Stereopreference of ROL toward triacylglycerols can be predicted by analyzing the geometry of the substrate docked to ROL: if the torsion angle Φ_{O3-C3} of glycerol is more than 150° , the substrate will preferably be hydrolyzed in sn-1, otherwise in sn-3. For ROL, the following rules were derived: (i) residue 258 affects stereoselectivity by steric interactions with the sn-2 substituent rather than polar interactions. To a lower extent, **stereoselectivity** is influenced by **mutations** further apart (L254) from residue 258. (ii) With "rigid" substrates, increasing the size of the binding site (**mutations** L258A and L258S) shifts **stereoselectivity** of hydrolysis toward sn-1, decreasing its size (L258F and L258F/L254F) toward sn-3.

L12 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

1998:243909 Document No. 129:37944 Directed evolution of an esterase for the stereoselective resolution of a key intermediate in the synthesis of epothilones. Bornscheuer, Uwe T.; Altenbuchner, Josef; Meyer, Hartmut H. (Institute for Technical Biochemistry, University of Stuttgart, Stuttgart, 70569, Germany). Biotechnology and Bioengineering, 58(5), 554-559 (English) 1998. CODEN: BIBIAU. ISSN: 0006-3592. Publisher: John Wiley & Sons, Inc..

AB The directed evolution of an esterase from *Pseudomonas fluorescens* using the mutator strain Epicurian coil XL1-Red was investigated. Mutants were assayed for their ability to hydrolyze a sterically hindered 3-hydroxy ester, which can serve as a building block in the synthesis of epothilones. Screening was performed by plating esterase producing colonies derived from mutation cycles onto minimal media agar plates containing indicator substances (neutral red and crystal violet). Esterase-catalyzed hydrolysis of the 3-hydroxy ester (Et

or glycerol ester) was detected by the formation of a red color due to a pH decrease caused by the released acid. Esterases isolated from pos. clones were used in preparative biotransformations of the Et ester. One variant containing two **mutations** (A209D and L181V) **stereoselectively** hydrolyzed the Et ester resulting in 25% ee for the remaining ester.

L12 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

1994:695932 Document No. 121:295932 Rational reconstruction of the active site of a class Mu glutathione S-transferase. Shan, Shu-ou; Armstrong, Richard N. (Dep. Chem. Biochem., Univ. Maryland, College Park, MD, 20742, USA). Journal of Biological Chemistry, 269(51), 32373-9 (English) 1994. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB Isoenzymes 3-3 and 4-4 of the mu class glutathione S-transferases share 77% sequence identity but have distinctly different catalytic properties. Anal. of the crystal structure of isoenzyme 3-3 in complex with the diastereomeric products of the addition of GSH to phenanthrene 9,10-oxide (Ji, X., Johnson, W. W., Sesay, M. A., Dickert, L., Prasad, S. M., Ammon, H. L., Armstrong, R. N., and Gilliland, G. L. (1994) Biochem. 33, 1043-1052) reveals that three residues that are in van der Waals contact with the xenobiotic portion of the product are different in the type 4 subunit. The three mutations, V9I, I111A, and S209A have been introduced into isoenzyme 3-3 individually and in combination to minimally reconstruct active site of the enzyme to mimic the type 4 subunit in structure and function. The results suggest that the V9I **mutation** is an important determinant in the **stereoselectivity** of the enzyme toward enones and epoxides. The I111A mutation increases the catalytic efficiency of the enzyme toward para-substituted 4-phenyl-3-buten-2-ones (XPBO) as measured by kcat/KmXPBO but does not affect kcat. The S209A mutation has no effect on catalysis. The double and triple mutants V9I/I111A and V9I/I111A/S209A exhibit both a high stereoselectivity and high kcat/KmXPBO comparable to that of isoenzyme 4-4. Anal. of substituent effects on the kinetics and stereoselectivity of the enzyme toward the enone substrates suggests that the mechanistic bases for the catalytic behavior of the isoenzyme 4-4 and the reconstructed mutants are not identical. The results provide functional evidence for the catalytic importance of specific residues previously identified by x-ray crystallog.

L12 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

1993:186803 Document No. 118:186803 Chiral recognition at cytochrome P450 1A2 active site: Effects of mutations at the putative distal site on the bindings of asymmetrical axial ligands. Krainev, Arkadi G.; Shimizu, Toru; Ishigooka, Masako; Hiroya, Kou; Hatano, Masahiro (Inst. Chem. React. Sci., Tohoku Univ., Sendai, 980, Japan). Biochemistry, 32(8), 1951-7 (English) 1993. CODEN: BICHAW. ISSN: 0006-2960.

AB Effects of mutations at the putative distal site of cytochrome P 450 1A2 on chiral discrimination for binding (R)-(+)- and (S)-(-)-1-(1-naphthyl)ethylamine (ligand I), (R)-(-)- and (S)-(+)-1-cyclohexylethylamine (ligand II), and (R)-(+)- and (S)-(-)-1-(4-pyridyl)ethanol (ligand III) were studied by optical absorption spectra. The wild-type P 450 1A2 exhibited different dissociation consts. (Kd) for the R- and S-enantiomers of these ligands. The R/S ratios of the Kd values for ligands I and II were 5.2 and 2.9, resp., and the S/R ratio for ligand III was 6.0. Mutations at the putative distal site, such as Glu318Asp and Glu318Ala, remarkably enhanced the discrimination: the R/S ratio of the Kd values for ligand I increased from 5.2 to 20-60, while the R/S ratio for ligand II decreased from 2.9 to 0.8-0.9. These remarkable changes in the R/S ratios were not observed with Glu318Asp mutation for ligand III binding, whereas affinities for both enantiomers of ligand III were markedly decreased by the Glu318Ala mutation. Mutation

Thr319Ala increased the R/S ratio of the K_d values for ligand I slightly but markedly decreased the R/S ratio of ligand II (from 2.9 to 0.8) and the S/R ratio of ligand III (from 6.0 to 1.0). Similar enhancements of the chiral discriminations were observed with the mutation Lys250Leu at another putative substrate-recognition site. Differences between the R- and S-enantiomers of the standard enthalpy and entropy of ligand III binding were changed most remarkably by the Thr319Ser mutations. From these findings, together with other spectral data, it is suggested that (1) Glu318 and Thr319 play important roles in the chiral recognition of asym. axial ligands, (2) Thr319 contributes thermodynamically to the discriminations of those chiral axial ligands, and (3) Lys250 is important in the chiral recognition and may be located close to a ligand access channel and/or a substrate-recognition site of this enzyme.

L12 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2004 ACS on STN

1991:674409 Document No. 115:274409 Studies of the catalytic mechanism of an active-site mutant (Y14F) of Δ^5 -3-ketosteroid isomerase by kinetic deuterium isotope effects. Xue, Liang; Talalay, Paul; Mildvan, Albert S. (Sch. Med., Johns Hopkins Univ., Baltimore, MD, 21205, USA). Biochemistry, 30(45), 10858-65 (English) 1991. CODEN: BICHAW. ISSN: 0006-2960.

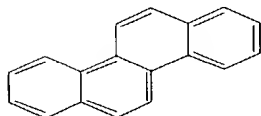
AB Δ^5 -3-Ketosteroid isomerase (EC 5.3.3.1) from *Pseudomonas testosteroni* catalyzes the conversion of androst-5-ene-3,17-dione to androst-4-ene-3,17-dione by a stereoselective transfer of the 4 β proton to the 6 β -position. The rate-limiting step is the concerted enolization of the enzyme-bound substrate, comprising protonation of the 3-carbonyl O atom by Tyr-14 and abstraction of the 4 β proton by Asp-38. Primary, secondary, solvent, and combined kinetic deuterium isotope effects were used to investigate the mechanism of the Y14F mutant, which lacks the proton donor and is 104.7-fold less active catalytically than the wild-type enzyme. With [4 β -D]androst-5-ene-3,17-dione as a substrate in H₂O, a lag in product formation was observed which approached, by a 1st-order process, the rate observed with protonated substrate. With the protonated substrate in D₂O, a burst in product formation was detected by derivative anal. of the kinetics data which approached the rate observed with the 4 β -deuterated substrate in D₂O. The absence of such lags or bursts with the protonated substrate in H₂O or with the 4 β -deuterated substrate in D₂O, as well as the detection of buffer catalysis by phosphate at pH 6.8, indicated that one or more intermediates dissociated from the enzyme and partitioned to substrate 31.6-fold faster than to product. When corrected for these exchange effects, the k_{cat} and k_{cat}/K_m values both showed a primary kinetic isotope effect of 2.4 for the 4 β -D substrate. The detection of a secondary kinetic isotope effect on k_{cat}/K_m of 1.6 with the 4 α -D substrate and the absence of an inverse secondary kinetic isotope effect with the 6-D substrate (1.02) indicated that enolization was rate-limiting for the Y14F mutant. The primary kinetic isotope effects on k_{cat}/K_m with the 4 β -D substrate of 2.33 found in H₂O decreased to 1.16 in D₂O, and the solvent isotope effect of 7.69 observed with protonated substrate decreased to 3.85 with the 4 β -D substrate, establishing a stepwise enolization mechanism. A minimal mechanism of the reaction catalyzed by the Y14F **mutation** thus involves the initial **stereoselective** removal of the 4 β -proton by Asp-38 to form the dienolate carbanion intermediate, which dissociates from the enzyme and is protonated in solution either at C-4 to regenerate the substrate or more slowly on the C-3 oxyanion to form the dienol, which reketonizes rapidly to form the product. Comparison of k_{off} of the intermediate from the Y14F mutant with that found with the D38N mutant indicated that the phenolic OH group of Tyr-14 contributes at least 7.6 kcal/mol to the free energy of binding of the intermediate. Tyr-14 thus appears to play a major role not only in the formation of the dienolic intermediate but also in binding it tightly to the

enzyme. A reaction coordinate free energy contour diagram was used to compare the concerted enolization mechanism catalyzed by the wild-type enzyme with the stepwise carbanion mechanism catalyzed by the Y14F mutant and the stepwise oxycarbonium ion mechanism catalyzed by the D38N mutant.

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1983:192719 Document No. 98:192719 Stereoselectivity in the metabolism, mutagenicity and tumorigenicity of the polycyclic aromatic hydrocarbon chrysene. Vyas, K. P.; Yagi, H.; Thakker, D. R.; Chang, R. L.; Wood, A. W.; Levin, W.; Conney, A. H.; Jerina, D. M. (Lab. Bioorg. Chem., Natl. Inst. Arthritis, Diabetes, Dig. Kid. Dis., Bethesda, MD, 20205, USA). Polynucl. Aromat. Hydrocarbons: Phys. Biol. Chem., Int. Symp., 6th, Meeting Date 1981, 859-71. Editor(s): Cooke, Marcus; Dennis, Anthony J.; Fisher, Gerald L. Battelle Press: Columbus, Ohio. (English) 1982. CODEN: 49MZAE.

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AB A review with 25 refs. on the stereoselectivity in the metabolism, mutagenicity, and tumorigenicity of chrysene (I) [218-01-9].